Intrinsically Disordered Proteins (IDP) and Aggregates II

1137-Pos Board B88
Optimized Force Fields for Simulations of Intrinsically Disordered Proteins
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Some frequently encountered deficiencies in all-atom molecular simulations, such as nonspecific protein-protein interactions being too strong, and unfolded or disordered states being too collapsed, suggest that proteins are insufficiently well solvated in simulations using current state-of-the-art force fields. In order to address these issues, we make the simplest possible change, by modifying the short-range protein-water pair interactions, and leaving all the water-water and protein-protein parameters unchanged. We find that a modest strengthening of protein-water interactions is sufficient to recover the correct dimensions of intrinsically disordered or unfolded proteins, as determined by direct comparison with small-angle X-ray scattering (SAXS) and Förster resonance energy transfer (FRET) data. The modification also results in more realistic protein:protein affinities, and average solvation free energies of model compounds which are more consistent with experiment. Most importantly, we show that this scaling is small enough not to affect adversely the stability of the folded state, with only a modest effect on the stability of model peptides forming alpha-helix and beta-sheet structures. The proposed adjustment opens the way to more accurate atomistic simulations of proteins, particularly for intrinsically disordered proteins, protein:protein association, and crowded cellular environments.

1138-Pos Board B89
Adaptive Particle Simulations of Alpha-Synuclein Fibril Formation
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A number of intrinsically disordered proteins, including alpha-synuclein and beta-amyloid, are known to form amyloids in neurodegenerative diseases such as Parkison's and Alzheimer's diseases, respectively, indicating generic behaviour of this class of proteins. The accompanying large conformational transition, from disordered to a beta-sheet, makes it difficult and extremely time-consuming to study the aggregation process by standard simulation methods. We have developed a Lambda Dynamics technique in which the coarse-grained particles, representing sequences of consecutive amino acids, respond to their environment by changing shape and interaction properties [1]. The evolving states of the particles are determined by internal and external interactions. The translational and rotational motion of the anisotropic particles are simulated with a newly developed concise Brownian Dynamics algorithm [2,3]. We present results on the aggregation of solvated spherical, disordered proteins into fibrils of elongated, beta-sheet forming proteins.
[1] I.M. Ilie, W.K. den Otter and W.J. Briels, in preparation
[2] I.M. Ilie, W.J. Briels and W.K. den Otter, in preparation
[3] I.M. Ilie, W.K. den Otter and W.J. Briels, J. Chem. Phys. 141, 065101 (2014)

1139-Pos Board B90
What does Evolution Tell us about the Structure of a Functional Amyloid Protein?
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Amyloids are insoluble fibrillar protein aggregates. While they are commonly found in human diseases, it is becoming increasingly clear that this type of structure is essential for a range of biological functions, with prominent examples in organisms ranging from bacteria to human. Functional amyloid and pathogenic amyloid share similar physical and chemical properties. Unlike pathological amyloids, however, the structures of functional amyloids are formed by polypeptide sequences whose amyloid structure has been under a positive evolutionary selection pressure. This important distinction provides us with an opportunity to obtain structural insights from an unexpected source: the covariation of amino acids among sequences within the same family of a functional amyloid protein. There is a long history for the idea of using coevolution for molecular structure prediction, but recent growth in sequence databases and new, efficient algorithms to disentangle indirect couplings in a network, have dramatically improved our ability to predict residue-residue contacts. We used recently developed sequence analysis methods (EVcoupling, PSICOV and GREMLIN) to extract distance restraints from a multiple sequence alignment of a functional amyloid protein. Together with an efficient force field, these restraints allow us to determine atomic resolution structural models. We find that the protein forms a beta-helical structure, where each turn corresponds to previously identified repeat sequences. The proposed structure is validated by previously published solid-state NMR, electron microscopy and X-ray diffraction data, and confirms an earlier proposed model derived by complementary means. To our knowledge, this is the first time the analysis of correlated mutations and computer simulations have been used together to study the structure of a functional amyloid. The current study therefore serves as a probe into the potential applicability of the approach in this domain.

1140-Pos Board B91
Enabling Biophysical Characterization of Intrinsically Disordered Protein Ensembles
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Elucidating structural details of protein conformations and its relation to protein function is a forefront area in structural biology. In this project, we will investigate nuclear co- activator binding domain (NCBD), an intrinsically disordered protein (IDP) implicated in acute myeloid/lymphatic leukemia (AML/ALL), which has the propensity to adopt extended conformations in unbound form and undergoes synergistic folding with substrate specific conformations when bound. Since IDPs like NCBD are highly flexible, their full conformational range is not observable by any one structure determination technique. We have developed a novel, integrated experimental and computational technique to elicit high-resolution structural details of such IDP ensembles. Specifically, we have (1) developed methods to prepare amino-acid-type selectively deuterated NCBD and utilize SANS contrast variation techniques to refine high-resolution conformational ensembles; (2) constructed parallel ensemble simulation strategies on heterogeneous computer architectures to generate millisecond timescale atomistic simulations; and (3) designed Bayesian inference techniques for statistical characterization of IDP conformational ensembles. The integrated approach enables accurate understanding, simulation and prediction of recognition mechanisms of NCBD under physiological condition.

1141-Pos Board B92
Developing a Protocol for Ensemble and Vibrational Probe-Containing Molecular Dynamics Simulations of the Nipah Ntail-XD Complex
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Intrinsically disordered proteins (IDPs) prove difficult to characterize using typical protein secondary structure indicators, such as CD and NMR spectroscopy, because of their dynamic nature. Molecular Dynamics (MD) simulations can be adapted for use in highly dynamic systems such as IDPs through enhanced sampling techniques. This project involves the characterization of the "fuzzy" Nipah Virus nucleo- and phosphoprotein bound complex by using MD simulations and vibrational probes. In order to run MD simulations on IDPs, a viable structural ensemble must be generated; this can be done by varying the simulation temperature to overcome energy boundaries and exchanging high-energy structures with those generated at low temperature ("replica exchange"). Once an ensemble of structures is generated, shorter simulations can be run in which a site-specific thiocyanate probe is incorporated into each structure. Eventually, an infrared (IR) lineshape can be simulated from the conformational ensemble for each label site and directly compared to experimental data. Progress towards each step of this multi-step simulation protocol will be discussed, as well as the prospects for a hybrid spectroscopic-theoretical determination of the conformational distribution of "fuzzy" bound complexes.

1142-Pos Board B93
Using Chemical Shifts to Generate Structural Ensembles for Intrinsically Disordered Proteins with Converged Distributions of Secondary Structure
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A short segment of the disordered p53 transactivation domain (p53TAD) forms an amphipathic helix when bound to the E3 ubiquitin ligase, MDM2. In the unbound p53TAD, this short segment has transient helical secondary structure. Using a method that combines broad sampling of conformational space with re-weighting, it is shown that it is possible to generate multiple, independent structural ensembles that have highly similar secondary structure distributions for both p53TAD and a P27A mutant. Fractional amounts of transient helical

secondary structure were found at the MDM2 binding site that are very similar to estimates based directly on experimental observations. Structures were identified in these ensembles containing segments that are highly similar to short p53 peptides bound to MDM2, even though the ensembles were re-weighted using unbound experimental data. Ensembles were generated using chemical shift data (alpha carbon only, or in combination with other chemical shifts) and cross-validated by predicting residual dipolar couplings.

1143-Pos Board B94

IDPs that Fold Upon Binding: What is the Role of the Partner Protein?

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Many intrinsically disordered proteins perform their functions within the cell by binding to a partner protein, often forming a defined structure (such as a helix or an extended strand) when they bind. To date most studies of coupled folding and binding have centred on the disordered protein or peptide, giving little consideration to the folded partner protein. Our recent work with 'model' IDP systems reveals that the folded partner protein can play an unexpectedly important role in the binding process.

1144-Pos Board B95

From Macroscopic to Molecular Interfaces: How do they Alter Protein Conformation?

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Conformation of proteins and peptides are not solely determined by their sequence, but are also strongly dependent on the environmental conditions. In addition to factors like salt concentration or pH, the environment presented by the surrounding molecules also play a major role on the conformational behavior of proteins and peptides. In this study, by using molecular dynamics simulations, we perform a comparative study on the conformational behavior of LKalpha-14 peptides in bulk water vs. macroscopic and molecular interfaces. The molecule under study is designed to have an alpha-helical conformation with the sequence (LKKLLKL)2. Our replica exchange simulations show that, similar to intrinsically disordered proteins, this molecule lacks a unique conformation when isolated in bulk water. However, in the presence of an air/water interface the molecule uniquely adopts the alpha-helix conformation. Further more, such a stabilizing effect is not limited to macroscopic interfaces, but can also be seen in the presence of molecular interfaces presented by surrounding molecules. Even when they are in a disordered state, molecules in solution display temporary molecular interfaces as a result of segregation of their hydrophobic and hydrophilic residues. Our simulations show that, these temporary molecular interfaces act as molecular chaperons in both accelarating the folding of peptides and also stabilize the alpha helical conformation when it forms. In light of these results, LK peptide can be identified as a classic example for conformational selection model.

1145-Pos Board B96

An Evolutionary Algorithm for the Design of Different Degrees of Secondary Structure in Intrinsically Disordered Proteins (IDPs)

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In recent experimental studies of coupled folding and binding of IDPs investigators have adapted methods from the protein folding literature whereby point mutations are used to identify the degree to which different regions are prefolded prior to the rate limiting step in binding and folding. These approaches assume a one-to-one correspondence between coarse-grained readouts of structure such as alpha helicity and the degree of foldedness. Our previous work demonstrated a lack of correlation between alpha-helicity and the degree of disorder within unbound IDPs. Therefore, an important first step toward mutagenesis based approaches for dissecting the mechanisms of coupled folding and binding is the design of sequences that (a) have a target alpha helicity (b) have a prescribed degree of disorder and (c) retain the residues corresponding to the binding interface. We report results from an evolution algorithm (EA) that designs sequences to meet the specified criteria. We coupled this sequence design algorithm to atomistic simulations based on the ABSINTH model to quantify residue-specific helicities for each sequence. We demonstrate our approach using the sequence of PUMA, which folds upon binding to its target protein MCL-1. In the unbound ensemble, PUMA forms two uncorrelated helical segments that partition the sequence into two halves. In the bound complex, PUMA forms a single long helix. Our design strategy successfully generates sequences that span the spectrum of conformational options for the unbound PUMA that includes sequences with uncorrelated helical segments N- and C-terminal and sequences with a single helix that spans the entire sequence length. Our approach is well suited to the design of sequences for use in experiments geared toward dissecting the mechanisms of coupled folding and binding of IDPs.

1146-Pos Board B97

CIDER: Classification of Intrinsically Disordered Ensemble Regions

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Intrinsically disordered proteins (IDPs) and intrinsically disordered regions (IDRs) of proteins fail to fold into well-defined three-dimensional structures as autonomous units. These sequences play important functional roles in signaling pathways, transcriptional regulation, and RNA metabolism. Recent studies have uncovered the physical principles underlying the relationships between IDP / IDR sequences and the range of conformations they adopt. IDP / IDR sequences can be classified into distinct conformational classes based on their amino acid compositions. These classes reflect the sequence-encoded balance between solvent-mediated intrachain electrostatic repulsions and attractions. Specifically, the number and linear sequence distribution of oppositely charged residues partitions the space of IDP / IDR sequences into globules, chimeras of globules and coils, designable random coils, and semi-flexible rod-like conformations.

Here, we present CIDER (Classification of Intrinsically Disordered Ensemble Regions) [http://pappulab.wustl.edu/CIDER]. The CIDER webserver provides a rapid and intuitive computational route for designating the appropriate conformational class to a sequence and calculating a number of key parameters that enable inferences regarding conformational properties of IDPs / IDRs. CIDER is freely available and can be used online via a web server to achieve rapid annotation of sequence-disorder relationships for IDPs / IDRs. We also present a freely available non-web version (localCIDER), which we use to perform a high throughput proteomic level sequence analysis to uncover patterns that govern known phosphorylation sites within IDP sequences. The correlation between known phosphosites and the distribution and fraction of charged residues suggests that phosphorylation is used to modulate the underlying charge patterning to engender an expansion of the disordered sequence. This hints at the use of phosphorylation as a reversible switch to toggle IDPs between distinct conformation al classes.

1147-Pos Board B98

Intrinsically Disordered Protein: A Thermodynamic Perspective

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Protein intrinsic disorder is commonly viewed as a binary attribute. This paradigm has been exploited for development and interpretation of prediction algorithms, which often classify positions as either ''structured'' or ''disordered'' based on sequence information. This work proposes a different perspective, based on the free energy difference between the disordered and structured thermodynamic states of the protein. Although requiring the assumption that intrinsically disordered protein is simply destabilized structured protein, several puzzling aspects of disorder can be subsequently rationalized. Most important of these is the fundamental insight that a small stability difference results in a large change in population of conformational states, meaning that only a few kcal/mol of energy (obtainable from ligand binding or pH change) could transform a disordered protein to a structured one. Thus, intrinsically disordered proteins may actually be energetically ''poised to respond'' to facilitate biological function. Several lines of computational evidence, generated using the eScape algorithm, support such a view. First, residues experimentally annotated as disordered exhibited distributions of lower predicted stability than structured residues. Second, the relative magnitudes of the average stability differences were small and thus consistent with possible population shifts between ''structured'' and ''disordered'' upon stability perturbation. Third, these stability differences were significantly correlated with disorder propensity, side chain volume, and experimental hydrophobicity scales. Finally, the thermodynamic information was used to train moderately effective support vector machine predictors of disorder. Although these novel predictors would benefit from technical refinement, their initial effectiveness suggests that progress in identification and classification of intrinsic disorder could be achieved by viewing disorder as part of a free energy continuum. In this new framework, the binary description would be replaced by a physical picture: a context-dependent estimate of conformational population.